PROGRESS IN NEUROSCIENCE PINS

Seminar Series of the
Brain & Mind Neuroscience Institute
Weill Cornell Medical College (WCMC)
&
The Graduate Program in Neuroscience of
WCMC and Sloan Kettering Institute

Thursday, 1/18/18, 3:45 PM
Weill Auditorium (C-200)

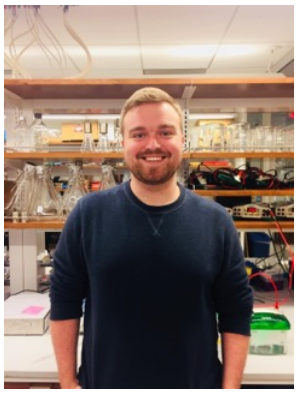
"Bright Monomeric Fluorescent Proteins with Rapid Expression and Cell-filling Properties for Neuronal Imaging"

Benjamin C. Campbell, PhD Candidate, Petsko Lab

We present new green and yellow FPs with dramatically enhanced brightness and maturation kinetics relative to EGFP, enabling significant improvements to neuronal imaging. The relatively low brightness of EGFP/EYFP often necessitates immunohistochemical enhancement or small-molecule dye injections to improve neuronal labeling, but these manipulations are laborious and increase background noise. We rationally engineered and screened libraries of folding-enhanced GFP mutants to develop mHyperGFP1, which is 3x brighter than EGFP, 3x faster-maturing, more pH-resistant, monomeric, and highly chemically stable. In mouse, AAV1-DIO-mHyperGFP1 illuminates dendritic spines within 3 days post-injection, in contrast to 7-14 days for EYFP. mHyperGFP1 highlights long-range projections 8x more effectively than EYFP and allows high-resolution monitoring of daily spine turnover in living mice using 2-photon microscopy. We also developed mHyperYFP1, which is brighter and faster-maturing than EYFP, and is remarkable for its ability to withstand chaotropic conditions relevant to tissue processing that denatured every other FP we tested, including Superfolder GFP. With intrinsic brightness and total cellular fluorescence intensity rivaling the best FPs currently available, our monomeric "Hyperfolder" FPs represent a much-needed upgrade to the FP toolkit and pave the way to a brighter future of fluorescence imaging in living animals.

"Chemical chaperones rescue mutant Munc18-1 dysfunction *in vitro* and *in vivo*"

Noah Guiberson, PhD Candidate, Burre Lab



Heterozygous mutations in Munc18-1, an essential regulator of neurotransmitter release, are linked to epilepsies, intellectual disability, movement disorders and/or neurodegeneration. These devastating diseases have a poor prognosis and no known cure. Using yeast, worm and mouse models, we found that at least five disease-linked missense mutations in Munc18-1 result in destabilization and aggregation of the mutant protein. Aggregates of mutant Munc18-1 incorporate wild-type Munc18-1, depleting functional Munc18-1 below hemizygous levels. Strikingly, we found that the chemical chaperones 4-phenylbutyrate, sorbitol and trehalose reverse the deficits caused by mutations in Munc18-1 in vitro and in vivo in multiple models, offering a novel strategy for the treatment of varied encephalopathies.

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